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AT

SYRACUSE UNIVERSITY

HUGH P. BAKER, Dean

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Technical Publication No. 37

ON THE BIOLOGY OF TRAMETES SUAVEOLENS (L.) FRIES

BY

RAY R. HIRT, Ph.D.

Assistant Professor of Forest Botany and Pathology

Published quarterly by the New York State College of Forestry at Syracuse, N. Y. Entered as second-class matter, Oct. 18, 1927, at the Post Office at Syracuse, N. Y., under the Act of Aug. 24, 1912.

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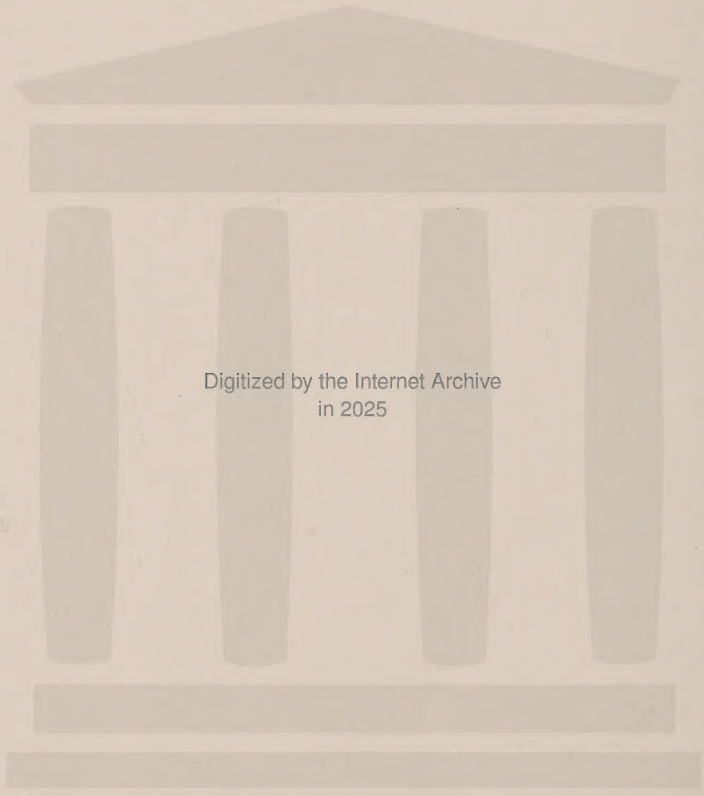

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ON THE BIOLOGY OF TRAMETES SUAVEOLENS (L) FRIES

RAY R. HIRT

Trametes suaveolens (L.) Fries has been known since the middle of the eighteenth century when it was first described by Linnaeus (18). It is found commonly upon *Salix* species (3, 5, 6, 7, 8, 14, 23, 26, 29) and occasionally upon *Populus* (31). Like so many fungi found only upon minor tree species, it has not attracted the attention of plant pathologists sufficiently for them to study it in detail. However, as the major tree species decrease in quantity, the minor species increase in importance and their pathogens accordingly receive attention. *Populus* and *Salix* species, within which *Trametes suaveolens* causes a white heartrot, are being used in increasing amounts for furniture, box-wood, excelsior, and paper pulp. Since the host woods of *Trametes suaveolens* are becoming of more economic importance, it was felt that information concerning the fungus and the decay of wood associated with it was desirable.

THE SPOROPHORE

Description

The pileus is sessile, 3–10 x 5–16 x 1–5 cm., white when young, gray to yellowish with age, azonate, glaborus to tomentose, margin thin and entire when mature; context white, corky, 0.5–2.5 cm. thick; tubes 0.3–2 cm. long, sometimes stratified, mouths white when young becoming yellowish with age, circular to slightly angled, 1–3 per mm., cystidia none; spores when fresh and mounted in water 4.5–10 x 2.5–4.1 μ;[1] hyphae of context and trama only slightly branched and with occasional clamp connections, 3.5–8 μ; odor of anise strong when fresh but lost upon drying.

Development

The fruiting-bodies develop on stubs of broken branches, in wounds on the trunk, and within hollow trunks. They appear during the early summer as nodular masses of white hyphae which rapidly take the form of normal sporophores with pores appearing as shallow pits on the under surface. As the sporophores increase in size the pit-like pores develop into tubes within which the hymenium is formed.

In young sporophores, the hyphae which form the upper surface of the context are loosely interwoven, regular in diameter,

[1] The average measurement of 500 freshly cast spores mounted in water was 3.5 x 8.7 microns.

thick walled, and collect in groups thus producing the tomentose effect of that surface. These surface-hyphae remain unmodified and continue to grow, becoming a part of the inner context as growth proceeds. A section taken through the context appears zonate. This is caused by alternate regions of densely and loosely interwoven hyphae, resulting probably from periodicity of growth. In producing the hymenophore the hyphae bend downward to form the trama, continuing the compact interwoven character of the inner context.

The hymenium consists only of the basidia upon which the basidiospores are produced. Basidia are present in all of the tubes of the hymenophore, even the youngest bordering directly upon the margin of the pileus. These basidia are closely appressed throughout the hymenium except near the mouth of the tubes where they are produced irregularly. In general the hymenium does not extend to the mouth of the tubes but instances have been observed where the hymenium of two adjacent tubes was continuous.

Usually sporophores of the previous season die before the following summer, apparently from excessive drying. Within hollow trunks the fruiting-bodies are often produced in large numbers and some of these show distinct stratification of the hymenophore, due in all probability to the fact that the air remains moist and the sporophores do not dry out but continue to develop for another season. This condition does not occur in sporophores produced on the outer side of the trunk, and hence stratified sporophores of *Trametes suaveolens* have not been commonly collected.

Sporulation

Spores are produced during the months of October, November, early December, and the first warm days of spring. The sporophores persist throughout the winter in a dormant condition, but with the coming of warm spring weather they revive for a limited period and produce spores.

Sporulation in the early spring occurs on warm days following cold nights. The spores are cast in great abundance for several days after which sporulation ceases. Alternate periods of high and low temperatures seem to be the factors influencing sporulation at this time. Sporophores brought into the laboratory during the months of January, February, March, and April were induced to sporulate as desired by subjecting them alternately to low and medium temperatures. Different sporophores were refrigerated at temperatures of 0°C,-2°C,-4°C,-6°C for 12 to 24 hours and then placed at temperatures of 20° and 22°C. Within 8 to 10 hours after exposure to the higher temperatures sporulation occurred, but any one sporophore could be stimulated to react in this way only 3 or 4 times after which vegetative growth of the hymenophore and context took place.

Basidia, which are about to produce spores, extend into the tubes slightly beyond the younger basidia. The sterigmata at first appear as blunt, teat-like processes, curving and becoming more pointed as they lengthen, and from the pointed tips the young spores appear as spherical bodies. When the spores enlarge to their normal size a single nucleus can be observed after proper staining.[1] The spores are attached to the sterigmata at the apicular end as noted by White (32) in his studies on *Fomes applanatus*. Enmass the spores are white; observed through the microscope they are hyaline. Viable spores are approximately uniform in size and devoid of vacuoles and oil globules.

Viability of Spores

Spores which were cast upon glass plates and upon heavy wrapping paper were stored both out of doors and in the laboratory. The temperature of the room in which the spores were kept varied from 39°C on hot summer days to 20°C during the winter months and the air was exceedingly dry. Daily tests of these spores gave about 50% germination for the first three weeks, after which tests were made once a week for 12 consecutive weeks with no appreciable variation in the viability of the spores. At the end of 3 months the tests were discontinued for a year, at which time further tests showed 25% of the spores were still viable. Thus it was shown that the spores of *Trametes suaveolens* retained their ability to germinate for at least 15 months when subjected to dessication and warm temperatures.

The spores from 5 collections placed out doors early in November lost their viability the latter part of December when the temperature reached –15°C. The spores of the remaining collections retained their viability until March, at which time the temperature dropped to –19°C, after which they would not germinate. Apparently *Trametes suaveolens* is dependent upon warm autumn days, quick spore germination, and rapid growth for propagation by means of basidiospores produced in the autumn.

SPORE GERMINATION AND CULTURE STUDIES

Methods

Spores were obtained from fresh sporophores found upon willow. The fruiting-bodies were brought into the laboratory where they continued to cast spores for 12 to 14 hours. Spore prints were

[1] The spores adhere to any surface with which they come in contact and this phenomenon was made use of in preparing permanent mounts of the spores. Spore prints were obtained upon clean slides and these slides with the adhering spores were placed in a killing and fixing solution, after which they were washed, and then treated as any paraffin mount from which the paraffin had been removed. After dehydrating, staining, clearing, and mounting, hundreds of the spores remained in place on the slides.

obtained upon sterile glass plates and these were stored in sterilized Petri dishes in the laboratory and out of doors. Germination tests were made in Van Tiegham cells and upon agar in Petri dishes. The latter method proved the more satisfactory.

Spore Germination

Germination occurred within 12 to 18 hours after planting the spores upon the agar. The most favorable temperature for rapid germination was 25°C. Spores planted upon agar and placed out doors (temperature about 7°C.) failed to germinate, but when brought into the laboratory and placed in the incubator, germination occurred as though the spores had just been planted. With a temperature favorable for germination it was possible within a few hours after planting to determine which spores would or would not germinate, since viable spores quickly swelled to about twice their normal size, whereas non-viable spores did not swell but became densely vacuolate and eventually collapsed. In general the germ tube appeared at the apex of the spore (opposite the apiculate end). Following the first germ tube, a second commonly appeared at the opposite end. The author has never observed two germ tubes appearing from the same spore simultaneously. Young germ tubes apparently have no influence upon the directional growth of neighboring ones as proved to be the case with the germinating spores of *Polyporus gilvus* (11). The germ tube develops directly into the primary mycelium which is characterized by the comparatively wide hyphae with few cross-walls and the absence of clamp connections. Measurements of the growth of these hyphae on agar (temperature 22°C) showed an average increase in length of 17μ per hour per hypha.

Where branches arise in either primary or secondary mycelium a slight constriction is visible (Fig. 17). Within 42 to 66 hours after planting the spores, both aerial and submerged hyphae were produced from the primary mycelium. The submerged hyphae are not of constant width but range from narrow to extremely wide; anastomosing is frequent and branching is varied (Figs. 33, 34, 35). The aerial hyphae arise directly from the wide superficial hyphae. They are narrow, quite free of septations and more or less twisted in their growth (Fig. 16). They commonly coil about one another forming cable-like structures (Fig. 15). Helicoid formations (Fig. 26) are common. At this stage the hyphae appear enmass as a fluffy, snow-white covering of the agar surface. After a lapse of several days raised masses of mycelium are noticeable. These gradually become pale yellow to light tan, comparable in color to the pore surface of mature sporophores. From the colored areas numerous honey-yellow (27) drops of liquid usually exude. In form and structure the hyphae of the colored patches do not differ from those of any other area. As the cultures grow

older the aerial hyphae become appressed to the agar and form a chamois-skin-like layer over the surface.

Secondary mycelium is characterized by the presence of clamp connections but in other respects is similar to the primary mycelium.

Swollen tip-cells (Fig. 31) are common to both primary and secondary mycelium. They are present in very young mycelium and continue to be produced in the oldest cultures. Since they are commonly found throughout the context of the sporophore and on the hyphae which occasionally grow over the cut surface of infected willow wood, it is manifest that they do not appear as the result of growing the fungus in culture. Upon exposure to dry air for a short period of time the swollen cells collapse and do not gain their turgidity when again placed in a damp chamber. These swollen cells are sufficiently characteristic of the fungus that they might be used as a partial means of identifying *Trametes suaveolens* in culture.

Secondary Spores

Young cultures originating from basidiospores often produce true oidia and clusters of oidia-like spores. Within 42 hours after cultures have been started from basidiospores, short aerial hyphae arise from the primary mycelium. These hyphae are usually narrower than the hyphae from which they originate but resemble them in other respects. After growing into the air for a short distance they swell out slightly at the tips (Figs. 39, 40, 43) and from these swollen tips several branches arise. Cross walls develop quickly (Figs. 40, 41, 42) and then the filaments rapidly become broken up into spores (Figs. 45, 46) which may vary from spherical to oval or cylindrical with rounded ends. Usually the development does not stop at this stage, for the tip cells bud to form clumps of spores (Figs. 40, 44) or the spores in the filament may bud (Fig. 49) to produce spherical or oval spore masses (Figs. 47, 48). The hyphae which bear clusters of spores break up as illustrated in Figures 51 and 52, in which it is seen that only the original hyphae remain intact. These spores germinate readily and repeat the process.

The spore clusters are similar to the bulbils of *Papulospora byssina* Hotson and *Papulospora nigra* Hotson as illustrated by Hotson (12) and to those of *Corticium alutaceum* (Schrad) Bres. shown by Lyman (15). Bulbils are considered as sclerotoid in nature, the cells of the mass being purely vegetative cells capable of resuming growth after a period of rest. Hotson found that in the older bulbils of *Papulospora pallidula* Hotson "the cells composing them adhere less firmly together, becoming more and more like independent spores," a few of which germinate and "which seem to act independently, like spores." This implies that between the cells of a bulbil some relationship exists whereby they ordinar-

ily renew growth only when in contact with one another. This is not true of the cells in the spore clusters of *Trametes suaveolens*, for the cells germinate readily when entirely isolated from each other and they reproduce the fungus, thereby meeting the definition of spores.

It is interesting to note the similarity in development between the bulbils and the spore clusters of *Trametes suaveolens*. Both may arise from a single hypha; they may develop from a single swollen tip cell by a process of branching (dichotymously or otherwise), budding, or a combination of both. They may originate from intercalary cells as well as terminal cells and in form may be irregular or spherical. Bulbils are usually colored but may be almost colorless on a clear substratum similar to the spore clusters of *Trametes suaveolens*. The two points of difference seem to be namely: (a) the spore clusters separate into individual cells (spores) without being disturbed whereas force is required to separate the cells in a bulbil; (b) the individual cells in the bulbil-like bodies are true spores whereas in true bulbils the cells reproduce the fungus only in mass or, at best, when loosely aggregated.

The first spores formed in a hypha prior to the production of a spore cluster are similar to oidia in their method of formation, possession of a thin wall, and their ability to germinate quickly to produce a primary mycelium. On the other hand they differ from oidia in that they can bud to form spores of a similar nature while still in position in the filament and thus form a spore cluster. The spores in the filaments and spore clusters have a rather definite size and shape, and in this respect they partake of the nature of conidia. It seems best however, to rank them as a type of oidia which may be borne in specialized clusters.

It is not unusual to find the spore clusters enclosed within drops of water similar to the oidial clusters of *Coprinus lagopus* described by H. J. Brodie (34). When this occurs the spores may completely fill a single drop and give the appearance of being closely appressed within a sporangium wall, or they may only partly fill the drop, similar to those of *Coprinus lagopus* illustrated by Brodie (34, Text figure 2). In either instance when the water droplets are touched with the point of a needle they burst and the released spores fall to the medium below. Since droplets of water without enclosed spores are exceedingly common at hyphal tips throughout both the primary and secondary mycelium, it does not appear that they are especially associated with the spore clusters of *Trametes suaveolens*.

During the production of spore clusters the cultures have a powdery appearance which lasts from 6 to 11 days after their first formation. Following this the cultures change to a fluffy, snow-white appearance and the spore clusters gradually disappear.

Chlamydospores (Fig. 66) occur infrequently in the mycelium and have only been observed in submerged hyphae.

Germination of Oidia

Oidia germinate within 6 hours after planting on agar. There is no swelling evident and the germ tube appears as an outward bulging of the wall. One or several germ tubes may be produced from a single spore and develop directly into primary mycelium similar in every respect to that developed in basidiospore cultures. Aerial hyphae appear within 20 hours after germination and 10 hours later spore clusters are fully developed. Thus it is seen that spore clusters are produced in less time from oidia than from basidiospores. From this point, cultures started from either kind of spores are identical in their development.

Anise Odor

Various experiments were performed in an attempt to associate the production of the characteristic anise odor with special structures in the mycelium, but without success.

During the experiments it was observed that where the aerial hyphae were not abundant, such as in cultures of the fungus on willow and poplar agar, the odor was very faint or entirely lacking. On Leonian's agar, carrot agar, or any other medium upon which the aerial mycelium developed abundantly the odor was strong. When the odor was very noticeable it was also observed that helicoid formations (Figs. 26, 28) were numerous. These formations were invariably surrounded with droplets of liquid, probably guttated from the tips of hyphae in the coils. Droplets were also common at the tips of hyphae not included within the coils (Figs. 29, 30). However, it was impossible to determine whether these droplets were in any way responsible for the production of the anise odor although they were most abundant when the odor was strongest. As the cultures dried the odor gradually diminished until it could not be detected.

The larger honey-colored droplets previously mentioned seemed to result from the fusion of the guttated droplets of the hyphae tips. When they first appeared they were colorless, but as they increased in size, the color became distinct. The anise odor is strongest when these colored drops are present on the mass of mycelium. These drops of liquid have a peculiar, bitter, astringent taste and hence are more than just guttated water or water of condensation. These drops also retained the anise odor for a short time after they were drawn off from the mycelium and placed in a covered container, even though the quantity of liquid was minute.

This suggested the possibility that an essential oil might have been responsible for the odor, especially in view of the fact that Zellner (33) had mentioned the presence of an essential oil in one of the fungi.

The context from a fresh sporophore and living mycelium of the fungus in culture were treated separately by means of indirect

steam distillation, with the result that an essential oil was demonstrated to be present. The anise odor was very strong in the distillate and was retained for a long period of time.

It seems reasonable to conclude that the droplets of moisture about the hyphae tips are to some extent responsible for the anise odor either through absorbing and retaining the odor given off by the hyphae, or by actually containing minute quantities of the oil at the time they are formed.

Tube Cultures[1]

The fungus was grown upon numerous synthetic media as well as solid cylinders of willow and poplar wood. Upon willow and poplar agar an amber colored (27) zone was invariably produced around the growing mycelium. This phenomenon did not occur in any of the other media.

The best growth took place on agar with a hydrogen-ion value of approximately 4.80. Liquid extracted from the hymenophore of an actively sporulating fruiting body gave a pH of 4.88 and from the context of the same sporophore a pH of 5.00.

The cultures on agar slants developed similarly to those previously described. The yellowish raised masses never developed into fruiting bodies although the cultures were kept for a year before drying out. Cultures upon sterilized blocks of willow and poplar wood were started from inoculum taken from the interior of fresh sporophores primarily to see if fruiting-bodies would be produced in culture. Within 14 months after the cultures were begun, small fruiting-bodies were formed, from the hymenophores of which viable spores were cast in sufficient quantity to form a white deposit on the side of the culture tubes nearest the bodies.

Heterothallism

Miss Mounce (16) states that "if, in any species of the hymenomycete, one finds . . . that clamp connections are not found in monosporus mycelia, but only in compound mycelia formed by the union of two mycelia presumably of opposite sex, then one is justified in concluding that the species is heterothallic." Tissue cultures from sporophores of *Trametes suaveolens* and from wood decayed by the fungus always showed a great number of clamp connections to be present. On the other hand the writer observed that clamp connections were lacking in monosporus cultures, which pointed toward the fact that the fungus might be heterothallic in nature.

Ten monosporus cultures were started from spores gathered from a single sporophore. After growing for several days upon agar in Petri dishes they were paired in every possible way on

[1]All cultures were started from single spores unless otherwise stated.

agar in culture tubes where they were permitted to grow for two months. The paired and unpaired mycelia were then carefully examined for the presence of clamp connections. The results of this examination are recorded in Table I.

Table 1. *Ten monosporus mycelia were paired in every possible manner. The presence of clamp connections in these cultures is designated by p, their absence by a.*

8	p	p	p	p	p	a	a	a	a	a
5	p	p	a	a	p	a	a	p	a	a
4	p	a	p	p	p	p	p	a	p	a
2	p	p	p	p	p	a	a	p	a	a
1	p	p	p	p	p	a	a	p	a	a
10	a	a	a	a	a	p	p	p	p	p
9	a	p	a	a	a	p	p	p	a	p
7	p	p	a	a	a	p	p	p	a	p
6	p	a	p	p	a	p	p	a	p	p
3	a	p	p	a	a	p	p	p	p	p
	3	6	7	9	10	1	2	4	5	8

All of the monosporus mycelia as well as some of the paired mycelia were free of clamp connections. Designating mycelium number 10 as a "plus" strain, and those mycelia which produce clamp connections when paired with it as representing a "minus" strain, we note that mycelia 3, 6, 7, 9, 10 fall in the "plus" group and 1, 2, 4, 5, 8, in the "minus" group. From Table 1 it is obvious that the pairing of mycelia designated as opposites in this arrangement are not consistent in the production of clamp connections and hence are not of strictly opposite sex. Cultures 5 and 6 are extremely variable, 4 and 7 less so, in their relationships with the other mycelia in spite of the fact that 5 and 6 when paired with each other produce clamp connections, as do 4 and 7. If these last 4 cultures are omitted from the series, the remaining 6 mycelia always pair with each other in such a manner that 1, 2, 8 appear to be of opposite sex from 3, 9, and 10.

The foregoing data indicates that *Trametes suaveolens* is heterothallic in nature, although the sex factors are complicated to the extent that the fungus is not bisexual.

Dr. Irene Mounce recently reported a homothallic form of *Trametes suaveolens* (17), consequently the author made several new series of single spore cultures. In one instance only did clamp

connections appear on the hyphae in a monosporus culture. Upon pairing the monosporus mycelia lacking clamp connections, results were obtained similar to those recorded in Table I.

During March, 1931, the author supplied Dr. Mounce with several collections of fresh spores from sporophores of *Trametes suaveolens* growing on Salix species at Syracuse, N. Y. Sometime later she submitted, in substance, the following report and very kindly authorized its use.

Spores from collection number 1 were spread in a drop of sterilized distilled water on the surface of lactose gelatine in a Petri dish (April 4, 1931). Twenty-one germinating spores were isolated the following day and grown in culture. One culture did not develop. The remaining 20 monosporus cultures were examined April 21, 1931, and one culture was found to have clamp connections. From the 19 monosporus cultures lacking clamp connections, 10 were paired in all possible ways. From collection number 2, 10 monosporus cultures were secured following the same procedure used in securing the 10 monosporus cultures from collection number 1. These were also paired in every possible manner. On May 11 and 12 both sets of pairings were examined[1] for the presence of clamp connections. The results of the examinations are recorded in Tables 2 and 3.

Table 2. Ten monosporus mycelia secured from collection number 1 were paired in all possible ways. The sign + designates the presence of clamp connections; the sign — indicates the absence of clamp connections.

	1	2	8	3	5	7	9	4	6	10
1	—	—	—	+	+	+	+	—		
2	—		—	+	+	+	+			
8	—	—	—	+	+	+	+	—	—	—
3	+	+	+	—	—	—	—	—	—	—
5	+	+	+	—	—	—	—	—	—	—
7	+	+	+	—	—	—	—	—	—	—
9	+	+	+	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	+
6	—	—	—	—	—	—	—	—	—	+
10	—	—	—	—	—	—	—	+	+	—

[1] The isolations and examinations were made by Miss Ruth Macre under the direction of Dr. Irene Mounce.

Table 3. Ten monosporus mycelia secured from collection number 2 were paired in all possible ways. The sign + designates the presence of clamp connections; the sign — indicates the absence of clamp connections.

	1	2	8	6	3	4	7	9	10	5
1	—	—	—	+	—	—	—	—	—	—
2	—	—	—	+	—	—	—	—	—	—
8	—	—	—	+	—	—	—	—	—	—
6	+	+	+	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	+
4	—	—	—	—	—	—	—	—	—	+
7	—	—	—	—	—	—	—	—	—	+
9	—	—	—	—	—	—	—	—	—	+
10	—	—	—	—	—	—	—	—	—	+
5	—	—	—	—	+	+	+	+	+	—

In corresponding with the author, Dr. Mounce commented on the above results as follows: "The results show quite conclusively that these 2 strains are heterothallic and quadrisexual and hence markedly different from the homothallic strain with which we were working last year."

DECAY OF WILLOW BY TRAMETES SUAVEOLENS

Field Studies

Trametes suaveolens is found associated with a white heartrot of willow and poplar. Within living trees it causes a heartrot only, in dead trunks it also destroys the sapwood. The final stage of decay is a typical white rot, comparatively dry, punky to corky, and has the anise odor characteristic of the fungus. In living trees which have been infected with the fungus for sometime, the change from apparently normal wood to badly decayed wood is quite abrupt. In the cross section of a living trunk the decayed area never occupies all of the heartwood but is separated from the sapwood by a ring of heartwood one-half to one centimeter wide, which in turn may or may not be separated from the badly decayed wood by a narrow colored band.

The fungus appears to attack the lower trunk first, seldom causing decay below the root collar, and then grows slowly upward into the branches. In longitudinal section of a live trunk two distinct stages of decay are evident, namely, incipient and extreme decay, with all gradations between the two. Incipient decay in its

earliest stage is evident by fine lines of bleached elements extending vertically through the heartwood. As these bleached zones enlarge, other lighter colored areas appear extending horizontally along the wood rays. Eventually the bleached pockets fuse and result in a contiguous area of discolored but firm heartwood. From this point to the final stage of decay the change is no longer evident through change of color, but rather through the decreasing strength of the wood, which offers less and less resistance to cutting and breaking.

Microscopic Studies

Willow is a soft, light, ring-porous wood of fine texture. The wood rays are heterogeneous in type (the marginal cells differing in shape from the included cells), uniseriate, and few to many cells high. The vessels are found singly and in pairs, those of the spring wood being slightly larger than those in the summer wood. Tyloses occur within the vessels of the late sapwood and in those of the heartwood. The last row of cells in each annual ring is composed of wood parenchyma. The remainder of the xylem consists of fiber tracheids. The majority of the fiber tracheids have three distinct walls with the exception of those bordering directly upon the wood parenchyma cells.

The first microscopical evidence of decay is the disappearance of the tertiary wall in the fiber tracheids. Following the digestion of this wall the secondary wall is attacked, which in turn is regularly dissolved outward toward the middle lamella. There is no noticeable decrease in the width of the walls of the other elements even in extremely decayed wood. In the final decay the walls of all the elements are filled with perforations made by the hyphae in passing from cell to cell. Wood which is badly decayed presents, in cross section, the appearance of a net work of middle lamellae, vessels, wood parenchyma cells, and wood rays.

Fungal hyphae are found abundantly in the different elements throughout the severely decayed wood but are less numerous in early stages of decay. Two kinds of hyphae are discernible, large hyphae with clamp connections and very fine hyphae upon which clamp connections were never observed. The latter hyphae are the more numerous but can only be definitely determined to be present when enmass. Probably the fine hyphae are the first to invade uninfected regions, since, in apparently sound wood from which tissue cultures of *Trametes suaveolens* were obtained, the presence of hyphae could not be detected by means of stained sections. If large hyphae had been present they would have been observed because of their size and the large perforations they would necessarily have made in the cell walls, since they do not become constricted in penetrating the wall substance as the hyphae of certain other fungi do (21).

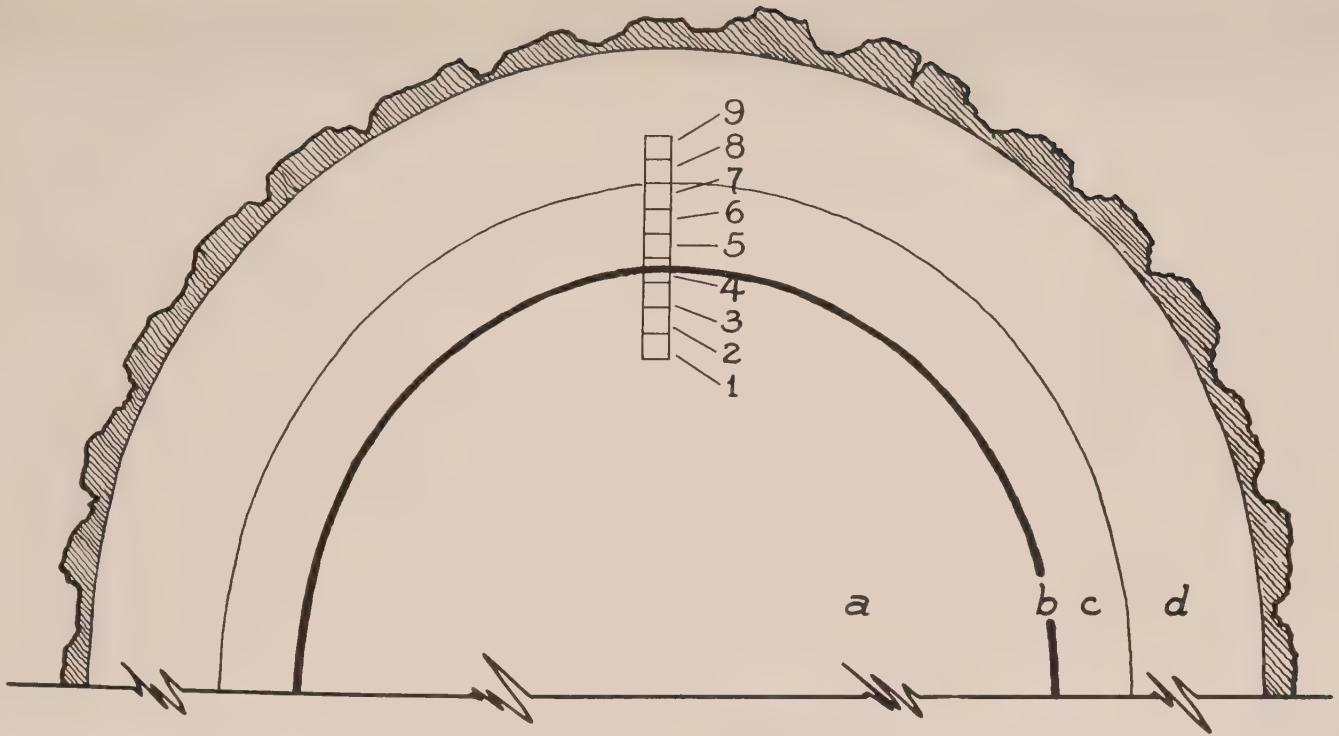


Fig. 1. *A cross section of a living willow trunk infected with Trametes suaveolens. (a) Decayed heartwood; (b) red-brown zone separating decayed heartwood from apparently sound heartwood designated by (c); (d) sapwood. The numbers indicate regions from which sections were obtained and from which cultures of the fungus were attempted for the purpose of locating the extent of the hyphae outward from the decayed core.*

Tissue cultures of *Trametes suaveolens* were obtained from the areas designated by the numbers (Text Fig. 1) 1 to 6 inclusive; from areas 7, 8, 9, no cultures could be secured. Microscopic examinations of sections taken from areas 1 to 9 inclusive showed hyphae only in sections from 1, 2, and 3. Hence, even though they could not be seen, there is no question but that hyphae were present in wood which appeared to be sound, namely, areas 4, 5, and 6. It is also obvious that hyphae did not extend into the sapwood, in fact did not extend entirely through the heartwood.

Microchemical Studies

Recent work (10, 11) tends to show that microchemical stains do not always give absolute proof of the chemical changes which occur during decay of wood. Harlow (10) clearly points out that phloroglucin-HCl reactions may lead to erroneous conclusions in some instances. It is probable, however, that when the typical pink or wine color results from applying phloroglucin-HCl to wood, it indicates the presence of lignin. If the typcial color reaction does not occur, one must not conclude that lignin is lacking, since only one component of the lignin may be responsible for the color reaction, and that may have been removed or changed by the fungal enzymes. The author is of the opinion that microchemical stains may be useful as indicators of the presence of lignin and cellulose, but that the stain reactions by no means establish a final proof of their presence or absence. In every case a chemical analysis is desirable as a check against the changes indicated by the stains.

Table 4. Normal heartwood of willow treated with phloroglucin-HCl and with chloroiodide of zinc.

Elements	Chloroiodide of zinc	Phloroglucin — HCl
Fiber tracheids	Tertiary wall stained blue	Secondary wall pink. Primary wall red.
Vessels	No stain reaction	Walls red. Tyloses red.
Wood parenchyma	No stain reaction	Secondary wall red. Primary wall red.
Ray cells	No stain reaction	Secondary wall red. Primary wall red.

Table 5. Decayed heartwood of willow treated with phloroglucin-HCl and with chloroiodide of zinc.

Elements	Chloroiodide of zinc	Phloroglucin — HCl
Fiber tracheids	Tertiary wall gone	Secondary wall gone. Primary wall red.
Vessels	No stain reaction	Walls red. Tyloses mostly gone, what remains is red.
Wood Parenchyma	No stain reaction	Secondary wall red. Primary wall red.
Ray cells	No stain reaction	Secondary wall red. Primary wall red.

Table 6. Normal heartwood of willow treated with 72% sulphuric acid.

Fiber tracheids	Tertiary wall dissolved, no residue visible. Secondary wall dissolved, no residue visible. Primary wall unaffected.
Vessels	Walls slightly swollen. Tyloses remained.
Wood parenchyma	Secondary wall dissolved. Primary wall unaffected.
Ray cells	Secondary walls swollen and mostly dissolved. Primary wall unaffected.

Table 7. Decayed heartwood of willow treated with 72% sulphuric acid.

Fiber tracheids	Any portions of secondary walls remaining are dissolved. Primary wall remained.
Vessels	No noticeable change.
Wood parenchyma	Any secondary wall remaining is dissolved. Primary wall remained.
Ray cells	Secondary walls swollen and mostly dissolved. Primary wall remained.

Phloroglucin-HCl indicates the presence of lignin in the primary and secondary walls of all the elements. The sulphuric acid treatment tends to show that the secondary walls of the fiber tracheids and wood parenchyma are cellulose in nature in that they dissolve with no visible evidence of a residue remaining. Chloroiodide of zinc stains the tertiary wall blue and this wall also dissolves in the acid. To sum up, sulphuric acid dissolves not only that which the microchemical stains indicate to be cellulose in composition, but also a portion which one interprets as being lignified when using phloroglucin-HCl, namely, the secondary walls of the fiber tracheids.

Both the acid and stains indicate that the middle lamella (the primary wall) of all the cells and the secondary walls of the vessels and ray parenchyma are highly lignified. It is interesting to note that the portions of the heartwood of willow remaining after being treated with 72% sulphuric acid correspond closely to those left after the wood has been decayed by *Trametes suaveolens*, hence one might conclude that the fungus utilizes to a great extent the carbohydrate content of the wood.

Inasmuch as the lamellae and the highly lignified walls of the elements remaining in extremely decayed wood contain many perforations, it is manifest that the fungus also secretes an enzyme which is capable of digesting lignin. That the perforations result from the hyphae dissolving their way through the walls, rather than by mechanically piercing them, seems obvious for the following reasons: (a) the hyphae form no appressoria prior to passing through the walls; (b) they do not decrease in diameter; (c) the perforations continue to enlarge after the hyphae have penetrated the walls.

Chemical Analyses [1]

The wood used for the analyses was that of *Salix pentandra* L. Sawdust from normal and decayed heartwood was ground very fine and sieved. The material which passed through a 60 mesh sieve but which was retained by an 80 mesh sieve was chosen for the analyses.

Table 8. Chemical analyses of normal and decayed heartwood of Salix pentandra L.

Component	Sound Wood			Decayed Wood		
	samples		Average	samples		Average
	1	2		1	2	
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Hot water soluble	9.72	9.66	9.69	9.08	9.15	9.11
Cold water soluble	7.60	7.44	7.52	5.75	5.69	5.72
Alkali soluble	25.60	25.82	25.71	28.16	27.94	28.05
Pentosans in wood	20.55	20.54	20.54	22.50	22.06	22.28
Lignin	22.94	23.07	23.00	26.88	26.81	26.84
Cellulose	47.47	47.15	47.31	38.24	37.83	38.03

[1] The analyses were made by Messrs. Rhodes and Moore under the direction of Dr. L. E. Wise, Professor of Forest Chemistry, New York State College of Forestry, Syracuse, N. Y.

Sound wood of a given volume was found to weigh 2.96 times as much as the volume of decayed wood. The loss in weight was 66.25% as calculated from the oven-dry weights of the samples taken.[1] All figures given in the results (Table 8) are based on the ash-free, bone-dry weights of the wood samples, and the ash-free, bone-dry weights of the isolated components or extracted wood. The ash figures of the wood samples were not considered reliable or typical of the wood because the ash content of the wood was affected to some extent by the grinding.

Table 9. The per cent by weight of the components in the decayed wood recalculated on the basis of unit volume.

Component	Per cent by weight of component in unit volume of sound wood	Per cent by weight of component in unit volume of decayed wood	Per cent loss by decay
Hot water soluble	9.69	3.07	68.3
Cold water soluble	7.52	1.93	74.4
Alkali soluble	25.71	9.48	63.2
Pentosans in wood	20.54	7.53	63.4
Lignin	23.00	9.07	60.6
Cellulose	47.31	12.84	72.8

The results of the analyses indicate that the fungus utilizes both the lignin and the cellulose components in its life processes. It must be borne in mind that these figures contrast normal wood and extremely decayed wood, and do not show the progressive changes taking place during the course of decay. It is probable that cellulose and lignin are not removed in the same proportion during incipient stages of decay as the figures indicate for the final stage. Earlier stages would probably show a higher percentage loss of cellulose since the unlignified tertiary walls are the first parts to be removed by the fungus. There is also a certain amount of fungus mycelium (fungus cellulose) present in the decayed wood, but what effect its presence might have upon the results obtained in the analyses is still an unanswered question. This particular problem is being studied by Dr. L. E. Wise, who is analysing the pure mycelium of *Trametes suaveolens*. However, it is apparent that the cellulose material is the chief source of food for

[1] In Table 9, the per cent weight of components in the decayed wood is recalculated on the basis of *unit volume* (rather than weight). This takes the loss of weight into consideration and gives comparable data.

the fungus, since it was present in greater quantity per unit of volume in normal wood, and decreased in greater proportion than lignin during decay.

Colored Zone Lines

A red-brown zone often separates the decayed from the apparently sound heartwood. This red-brown zone is made up of wood elements which are occluded with a brown deposit consisting, apparently, of partially digested cell wall substance. In the early formation of this zone, the tertiary walls of the fiber tracheids swell and change from hyaline to a faint amber color comparable with the amber zone in willow and poplar agar surrounding living mycelium of *Trametes suaveolens*. As the walls swell, they lose their identity and become a homogeneous mass, turning from amber to a deep dark red-brown and completely filling the lumina of the cells. In freshly cut wood the deposit is solid throughout the cell cavity but quickly checks upon drying. The brown deposit is present in the vessel cavities as well as in the fiber tracheids. Its presence in the vessels may be accounted for by an action on the tyloses similar to that on the tertiary wall of the fiber tracheids, or it may diffuse into the vessel cavities through the pits leading from the fiber tracheids. The writer was unable to demonstrate its formation within the vessel lumina. The fungal enzymes acting upon the wall substance are probably responsible for the change, and the brown material may represent the partially digested wall substance. This is insoluble in 72% sulphuric acid and did not become colored by any of the stains used; however, it is apparently available as food for the fungus since, on the side of the red-brown zone bordering extreme decay, the colored substance begins to break down due either to further digestion or to absorption by the hyphae. Remains of such zones a few millimeters back in the extreme decay show the larger hyphae ramifying throughout the brown substance in the cell cavities. The deposit is gradually broken down in an irregular fashion within the cell lumen until it entirely disappears, so that in the most extreme decay there is no trace of the red-brown substance. The author is of the opinion that in the willow wood studied, when brown lines occur during decay of such wood by *Trametes suaveolens*, they occur because the enzymes digest the cell substance in such quantity that this partially digested material cannot be absorbed by the fungal hyphae as rapidly as it is produced by the enzymes. Where the action is slower, namely where the apparently more-resistant secondary walls are acted upon after the tertiary walls are digested, no such brown lines are formed. The absence of these lines may also be due to the fact that the larger hyphae are present in such regions and are able to absorb the digested wall substance as rapidly as it is produced.

SAPROPHYTISM

There were no live cells in any portion of the heartwood studied, in fact, no living cells could be found in the oldest annual ring or two of the sapwood. Since the microscope did not reveal the presence of hyphae where it had been previously demonstrated that the fungus was present, the ability to obtain cultures was relied upon as an index to the advance of the mycelium. In living trees with the inner heartwood almost entirely decayed, there was always a narrow band of heartwood bordering the sapwood, and in no instance could positive cultures be obtained from the sapwood or the last few millimeters of the heartwood bordering directly upon the sapwood. Furthermore, trees infected with *Trametes suaveolens* which, ten years ago were entirely hollow, are still growing vigorously and have a solid shell of sapwood surrounding a narrow band of heartwood. Each year, one to several sporophores of the fungus are produced on the trunks, showing that the fungus is still alive within the wood.

Moreover, when sprouts are formed from old willow stumps rotted by the fungus, decay progresses upward into the sprouts only after a core of heartwood has been formed. From these facts it is logical to conclude that *Trametes suaveolens* is strictly saprophytic, inasmuch as its activities in living trees are limited wholly to the heartwood.

CONCLUSIONS AND SUMMARY

Poplar and willow are frequently planted in groups for ornamental purposes. Such groups may be protected from the attacks of *Trametes suaveolens* even though an infected tree or two may be present among them. The sporophores of the fungus develop during the summer but do not begin to sporulate until early fall, hence the fruiting bodies may be removed from the infected hosts prior to sporulation. In this way the infected trees may be retained if so desired without endangering the remainder of the group until such a time as they can be replaced with healthy trees.

Since the fungus grows only in the heartwood of living hosts, tree surgery, if properly done, would be successful in saving and improving the appearance of recently infected but desirable ornamental willows and poplars.

In stands of poplar or willow it is desirable to remove all infected trees since the fungus is able to spread rapidly because of the quick germination of basidiospores, production of viable secondary spores, and rapid growth of the mycelium, which, once established, soon destroys the heartwood of the host. Inasmuch as the fungus is a saprophyte any practice which will diminish or eliminate the exposure of dead wood on a living host, will lessen proportionately the chances of infection.

Trees growing on low, moist sites are more often attacked by the fungus. Host species growing on higher land are somewhat protected from infection because of the lower humidity of the air, and the consequent poorer condition for spore germination.

The fruiting bodies of the fungus are readily recognized because of their white color and peculiar anise odor when fresh. In most cases the decay caused by *Trametes suaveolens* can be recognized even though fruiting bodies are not present, since the anise odor permeates the decayed wood, and is even retained for some time after the wood is air dried.

Basidiospores stored in the laboratory retained their viability for over 15 months.

Temperatures as low as -19°C seemed to destroy the viability of basidiospores stored outdoors, and hence in the vicinity of Syracuse, N. Y., the fungus is dependent upon warm, humid, autumn days, quick spore germination, and rapid growth of the young mycelium in order to establish itself on a new host by means of basidiospores produced in the fall of the year. Once the mycelium is established, the low winter temperatures merely cause the fungus to become dormant until the approach of warm weather, when mycelial growth is resumed.

Trametes suaveolens normally sporulates in the autumn but with the approach of warm weather the following spring, some sporophores revive for a short time and produce a crop of viable basidiopores.

Basidiospores germinate within 12 to 16 hours after planting and secondary spores may be produced 25 to 30 hours later.

In culture certain secondary spores are produced in clusters resembling bulbils but do not function as such.

A convenient method of staining basidiospores is suggested.

Trametes suaveolens is heterothallic but not strictly bisexual.

Sporophores were produced in cultures originating from tissue removed from the interior of fresh sporophores.

The fungus is strictly a saprophyte and in living trees attacks only the heartwood.

A chemical analyses of the normal and decayed heartwood of willow showed that both the lignin and cellulose were destroyed, but that the latter was removed in greater proportion.

The colored material responsible for the colored zone of decay appears to be partially digested cell wall substance, which is produced in certain areas more rapidly than the fungal hyphae can absorb it, and hence lends color to the region in which it collects.

LITERATURE CITED

1. Bavendamm, W.
   1928. Neue Unterschungen uber die Lebensbedingunen holzzerstörender Pilze. Jena.
2. Berkeley, M. J.
   1860. Outlines of British fungology. London.
3. Cooke, M. C.
   1871. Handbook of British fungi. Vol. I. London.
4. ..... and Quelet, L.
   1878. Hymenomycetum. London.
5. Eddelbuttel, H.
   1911. Grundlagen einer Pilzflora des ostlichen Weserberglandes und ihrer pflanzengeographischen Beziehungen. Ann. Mycologici 9:475,525.
6. Fries, E.
   1821. Systema mycologicum. Vol. I.
7. .....
   1824. Hymenomycetes Europei.
8. .....
   1828. Elenchus fungorum. Vol. I.
9. Gillet, C.
   1878. Les Champignons. Paris.
10. Harlow, W. M.
    1928. Contributions to the chemistry of the plant cell wall. N. Y. S. Col. For., Tech. Publ. 24.
11. Hirt, R. R.
    1927. The biology of *Polyporus gilvus* (Schw.) Fries. N. Y. S. Col. For. Tech. Publ. 22.
12. Hotson, J. W.
    1912. Culture studies of fungi producing bulbils and similar propogative bodies. Proc. Amer. Acad. Arts and Sci. 48:225–306.
13. .....
    1917. Notes on bulbiferous fungi with a key to described species. Bot. Gaz. 54:265–284.
14. Lloyd, C. G.
    1915. Synopsis of the section apus of the genus *Polyporus*. Cinn.
15. Lyman, G. R.
    1907. Culture studies on polymorphism of hymenomycetes. Proc. Bost. Soc. Nat. Hist. 23:125–209.
16. Mounce, I.
    1921. Homothallism and the production of fruit-bodies by monosporus mycelia in the genus *Coprinus*. Trans. Brit. Mycol. Soc. 7:198–217.
17. .....
    1930. Notes on sexuality in *Fomes pinicola* (Sw.) Cooke, *Fomes roseus* (Fr.) Cooke, *Polyporus Tuckahoe* (Gusson) Sacc. et Trott., *P. resinosus* (Schrad.) Fr., *P. anceps* Peck, *Lenzites saepiaria* Fr., *Trametes protracta* Fr., and *T. suaveolens* (L.) Fr. Proc. Can. Phytopath. Soc. 1:27–28.
18. Murrill, W. A.
    1908. North American Flora. Polyporaceae.
19. .....
    1922. A fragrant Polypore. Mycologia 14:46–47.
20. .....
    1905. *Polyporus suaveolens*. Bul. Tor. Bot. Club. 32:477–478.
21. Nutman, F. G.
    1929. Studies of wood-destroying fungi. 1. *Polyporus hispidus* Fries. Ann. Appl. Biol. 16:41–64.
22. Overholts, L. O.
    1914. The Polyporaceae of Ohio. Ann. Mo. Bot. Gard. 1:140.

23. Overholts, L. O.
1915. The Polyporaceae of the middle-western United States. Wash. Univ. Series. 3:68.
24. Rabenhorst, L.
1844. Deutschlands Kryptogamen flora. 1:415.
25. Rea, C.
1922. British Basidiomycetes. London.
26. Rhoads, A. S.
1921. Some new or little known hosts for wood-destroying fungi. Phytopath. 11:326.
27. Ridgway, Robt.
1912. Color standards and nomenclature. Wash.
28. Saccardo, P. A.
1838. Sylloge fungorum. Polyporeae 6:338.
29. Stevenson, J.
1886. British fungi. Hymenomycetes. 2:222.
30. Weir, J. R.
1914. Notes on wood-destroying fungi which grow on both coniferous and deciduous trees. Phytopath. 4:271–276.
31. . . . . .
1917. Montana forest tree fungi. Mycologia 9:129–137.
32. White, J. H.
1919. On the biology of *Fomes applanatus* (Per.) Wollr. Trans. Roy. Can. Inst. 133–174.
33. Zellner, J.
1907. Chemie der Höhern Pilze. Leipzig.
34. Brodie, H. J.
1931. The oidia of *Coprinus lagopus* and their relation with insects. Ann. Bot. 45:315–344.

EXPLANATION OF PLATES

Plate I

Fig. 1, 3, 5, 7, 9, 11. Camera lucida drawings of a germinating basidiospore taken at the following intervals: 10:00 A. M., 12:00 noon, 1:15 P. M., 2:15 P. M., 3:15 P. M., and 4:15 P. M.

Fig. 2, 4, 6, 8, 10, 12. Camera lucida drawings of another germinating basidiospore taken at the same intervals as for the preceding spore.

Plate II

Fig. 13. Camera lucida drawing of the first spore taken at 5:15 P. M.

Fig. 14. Camera lucida drawing of the second spore taken at the same hour.

Plate III

Fig. 15. A portion of the secondary mycelium showing the hyphae twisting about one another to form cable-like structures.

Fig. 16. A young hypha showing the twisted growth prior to uniting with another hypha to form a cable-like structure.

Fig. 17. A narrow aerial hypha arising from the larger hypha growing over the surface of the substratum.

Fig. 18. This peculiar form of growth is common to the submerged hyphae in cultures growing on Leonian's agar.

Fig. 19. One of the larger hyphae of the secondary mycelium.

Fig. 20, 21. Two young plants of *Trametes suaveolens* 26 hours after planting basidiospores upon agar.

Fig. 22. A basidiospore which did not germinate. Such spores become highly vacuolate and eventually collapse.

Fig. 23. A viable basidiospore just prior to producing a germ tube. Note that it has swollen to about twice the size of the non-viable spore.

Fig. 24. A basidiospore just germinating.

Plate IV

Fig. 25, 26, 27, 28. Helicoid formations found in the mycelium with which moisture globules are commonly associated.

Fig. 29, 30. Moisture globules are commonly found at the tips of hyphae and where short branches occur.

Fig. 31. Swollen tip cells are common to the mycelium in culture as well as that which appears on cut surfaces of infected willow wood.

Fig. 32. The swollen tip cells collapse upon drying and do not regain their former turgidity when again placed in a moist chamber.

Fig. 33, 34, 35. Frequent forms of anastomosing common to the primary and secondary mycelium.

Fig. 36. 37, 38. True oidia produced by the primary mycelium growing upon unfavorable media.

Plate V

Fig. 39. Early branching indicative of a spore cluster.

Fig. 40. Cross walls forming in the branches. One branch has advanced more rapidly than the others and has produced true spores throughout its length and at the tip is a young cluster.

Fig. 41, 42, 43, 46. Early stages in the formation of spore clusters.

Fig. 44, 45. Early budding of the tip cell to form a cluster.

Fig. 45b, 47, 50. Mature spore clusters.

Fig. 48. Spore cluster formed from an intercalary cell.

Fig. 49. The first formed spores in a hypha budding to form a spore cluster.

Fig. 51, 52. Mature spore clusters broken up showing each cell as a separate spore capable of reproducing the fungus.

Plate VI

Fig. 53–61. Spores, from the clusters, germinating.

Fig. 62–64. Young mycelium produced 21 hours after planting spores from the sport clusters.

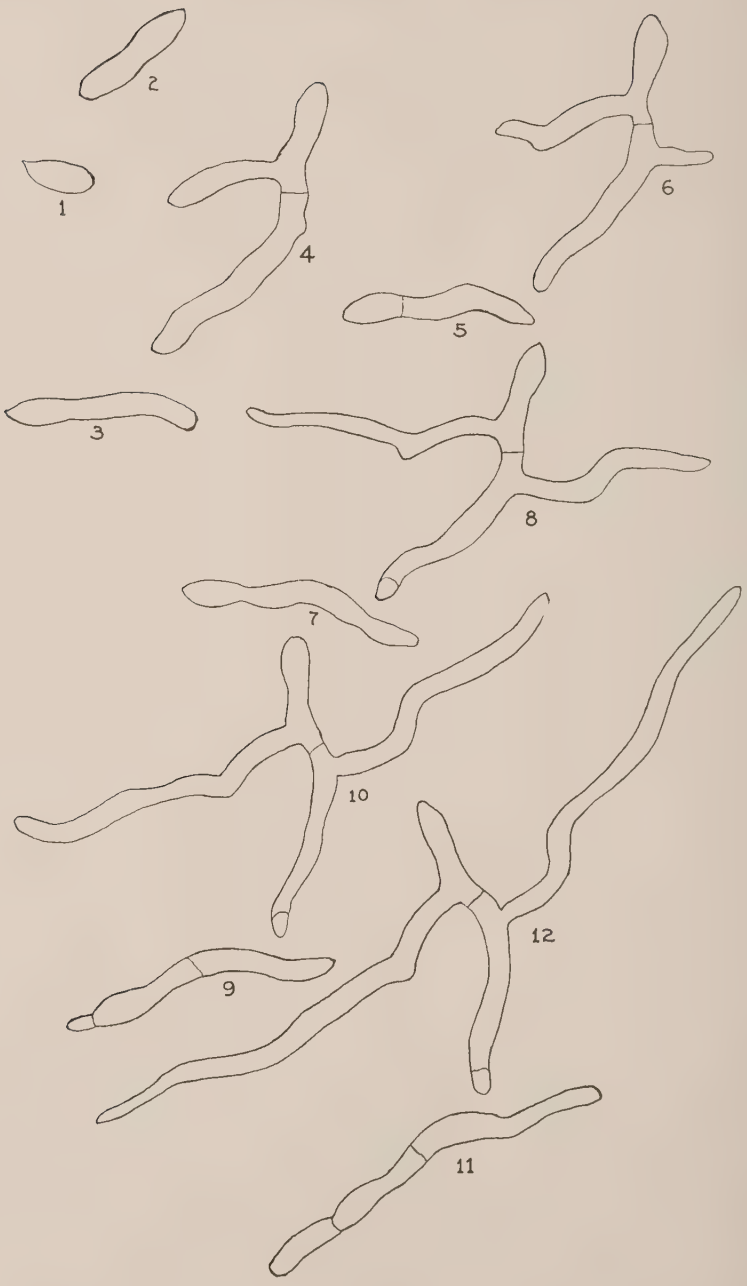
Fig. 65. A young spore cluster forming less than 30 hours after planting the secondary spores.

Fig. 66. Chlamydospores produced by the secondary mycelium.

Plate VII

Fig. 67. The cross-section of a willow trunk affected with heartrot caused by *Trametes suaveolens*. ½ Nat. Photographed by Henry Hopp and H. G. Harris.

Fig. 68. Sporophores of Trametes suaveolens growing on *Salix* species. ⅓ Nat. Photographed by Henry Hopp and H. G. Harris.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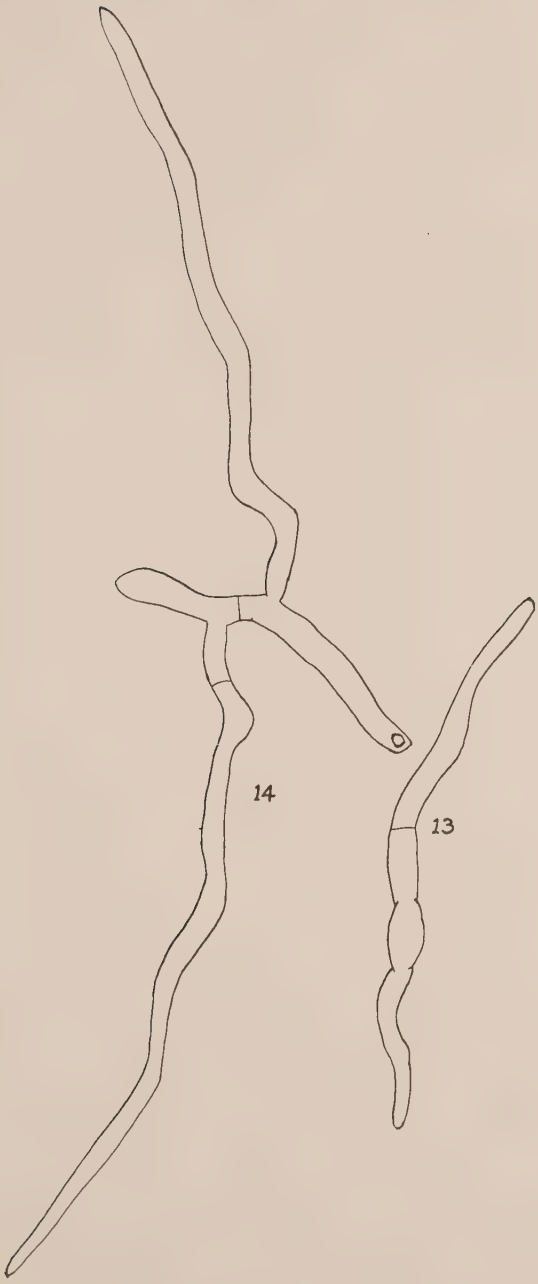
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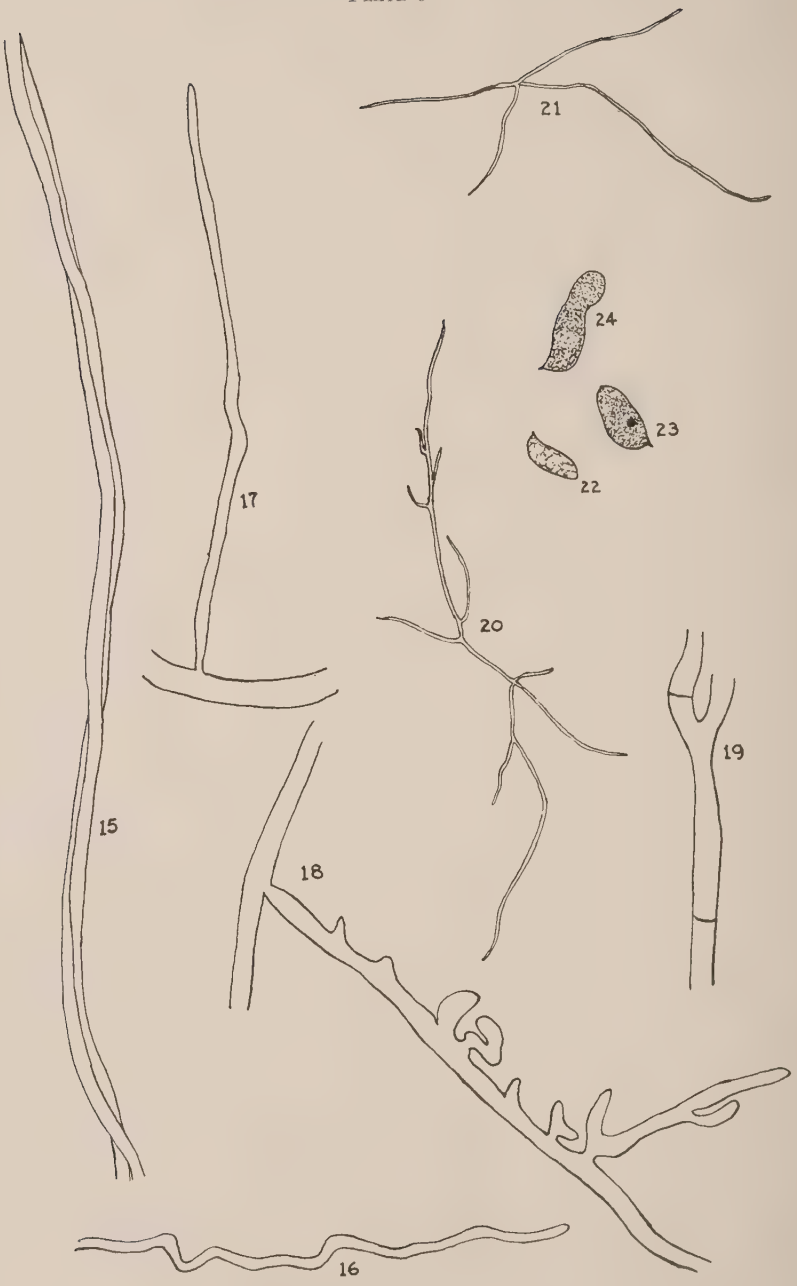
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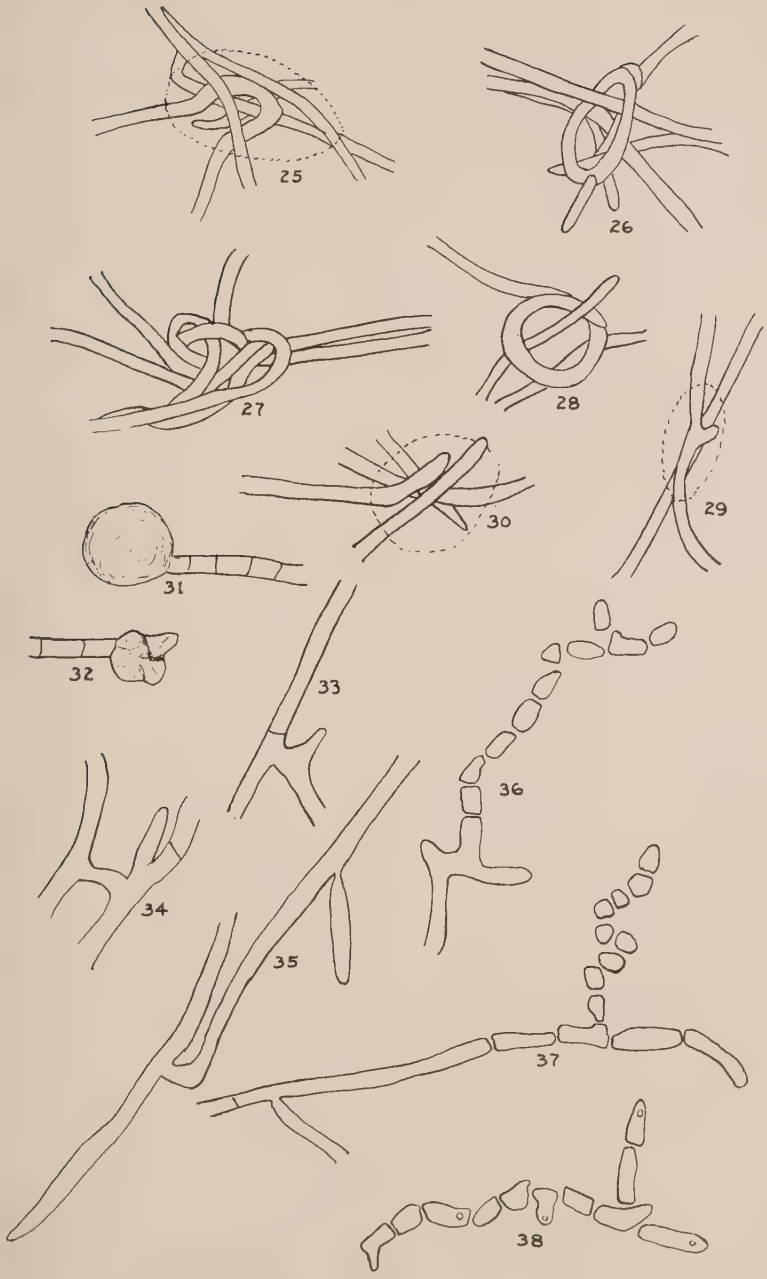
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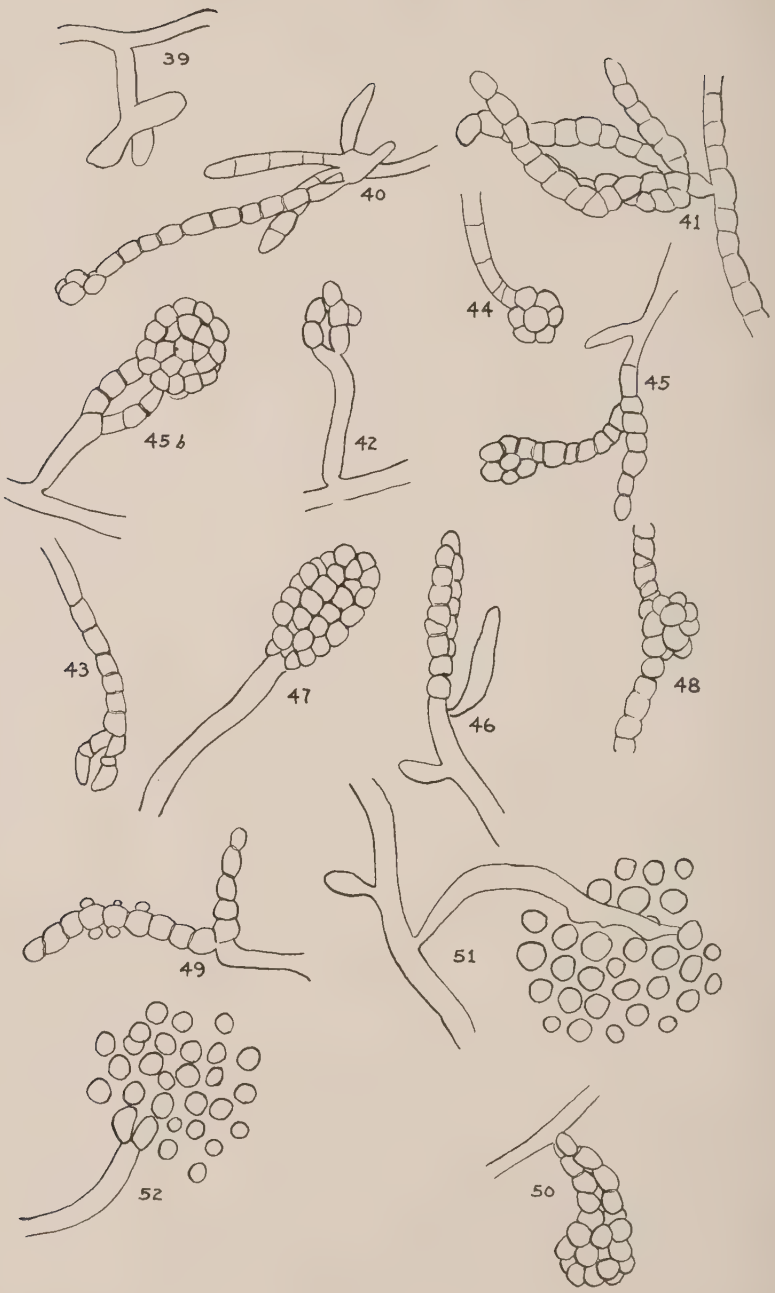
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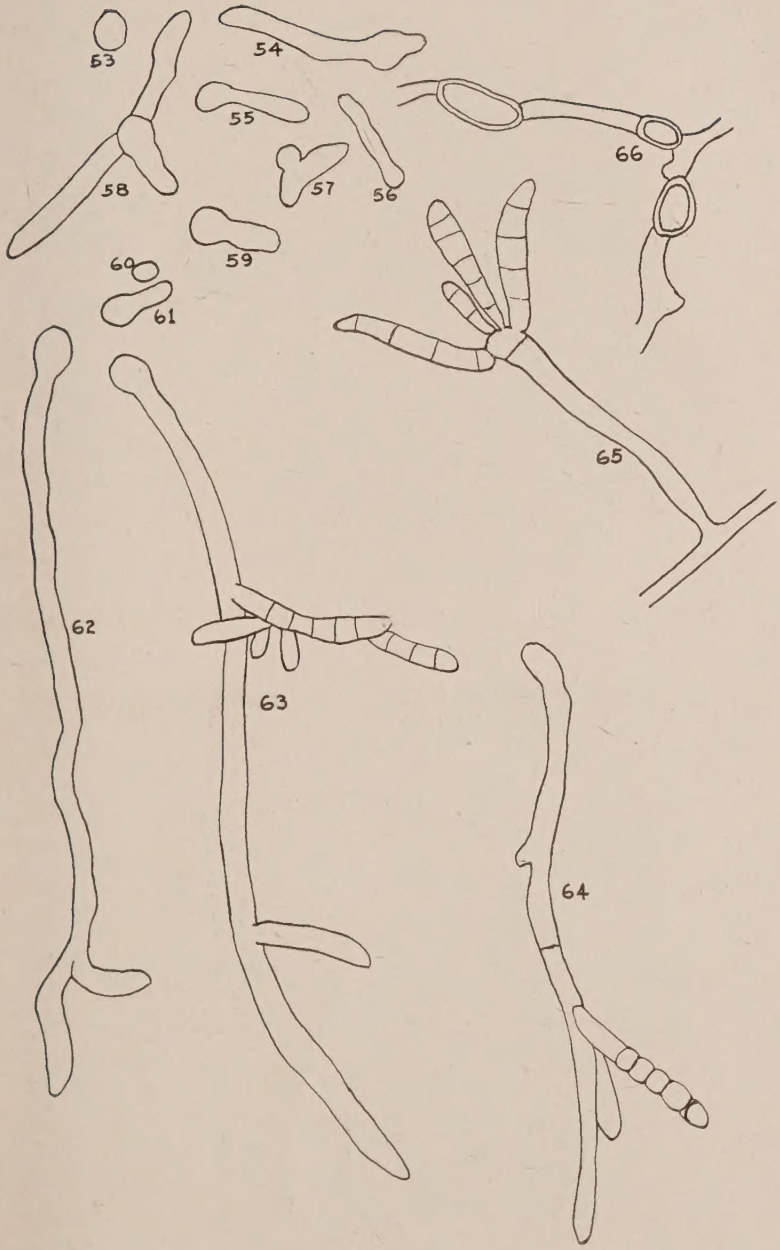
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